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PATENT
Docket No.: **176/60901 (6-11402-968)**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Shohei Koide	Examiner: Joseph F. Murphy
Serial No.	:	10/006,760	
Cnfrm. No.	:	2042	Art Unit: 1646
Filing Date	:	November 19, 2001	
For	:	METHOD OF IDENTIFYING POLYPEPTIDE MONOBODIES WHICH BIND TO TARGET PROTEINS AND USE THEREOF	

DECLARATION OF SHOHEI KOIDE UNDER 37 CFR § 1.132

I, SHOHEI KOIDE, declare as follows:

1. I am the inventor of the above-identified application.

2. I received a B.Sc. degree in Agricultural Chemistry from the University of Tokyo, Tokyo, Japan in 1986 and a Ph.D. degree in Agricultural Chemistry from the University of Tokyo, Tokyo, Japan in 1991. I was a postdoctoral Fellow at The Scripps Research Institute, La Jolla, California from 1991 to 1995.

3. I am currently an Associate Professor of Biochemistry & Molecular Biology at the University of Chicago, Chicago, Illinois. I have held my position at the University of Chicago since 2002. From 1995 until 2002, I was an Assistant Professor (1995-2001) and then an Associate Professor (2001-2002) of Biochemistry and Biophysics at the University of Rochester, Rochester, New York.

4. I am presenting this declaration to describe the high degree of conservation between mammalian tenth Fn3 domains of fibronectin and to explain that the references cited by the U.S. Patent and Trademark Office ("PTO") in the outstanding office action are irrelevant to the monobodies formed by modification of a mammalian tenth Fn3 domain of fibronectin.

5. The tenth Fn3 domain amino acid sequences from human (SEQ ID NO: 2 of the present invention), mouse (GenBank Acc. No. CAA63654), chimpanzee (GenBank Acc. No. XP_516072), dog (GenBank Acc. No. XP_536059), cattle (GenBank Acc. No. XP_879489), and rat (GenBank Acc. No. NP_062016) were aligned using the ClustalW program (http://www.ebi.ac.uk/clustalw/index.html#) on default settings. The results are attached hereto as Exhibit 1, and the β-strands are identified by letters A–G over the relevant amino acid residues. As shown in Exhibit 1, the mammalian tenth Fn3 domain is 86–100% conserved relative to the human Fn3 sequence of SEQ ID NO: 2. The β-strand sequences are highly conserved (β-strands A, F, and G) or identical (β-strands B, C, D, and E), and the loop region sequences are mildly conserved (loop region (E-F), highly conserved (loop regions B-C and C-D), or identical (loop regions A-B, D-E, and F-G). Because of the high degree of identity and conservation, particularly among β-strands, it is appropriate to consider the human Fn3 domain of SEQ ID NO: 2 as representative of mammalian tenth Fn3 domains generally.

6. Based on the structural homology, a person of skill in the art of molecular biology, protein biochemistry and/or protein engineering would fully expect mammalian tenth Fn3 domains that are highly similar to the human Fn3 domain of SEQ ID NO: 2 to be useful as a scaffold for preparing functional polypeptide monobodies that bind to a nuclear receptor of interest.

7. The outstanding office action cites several reference as evidence that (i) mammalian fibronectin is not conserved, and (ii) mutations to proteins are unpredictable. I respectfully disagree with both these conclusions for the reasons noted in paragraphs 8–10 below.

8. The PTO has cited Garcia-Pardo et al., "Primary Structure of Human Plasma Fibronectin," *J. Biol. Chem.* 260(18):10320–10325 (1985) ("Garcia-Pardo") as evidence that mammalian fibronectin is structurally diverse. The teachings of Garcia-Pardo are irrelevant to the claimed invention because the region of fibronectin that was analyzed by Garcia-Pardo contains the C-terminal 31-kDa domain. This region is distinct of the tenth Fn3 domain that is used as a starting scaffold for preparing the claimed monobodies. That is apparent when reviewing Figure 3 of Garcia-Pardo; the tenth Fn3 domain of SEQ ID NO: 2 is not present in Figure 3 (compare SEQ ID NO: 2 of the present invention with the 31-kDa domain shown in Figure 3 of Garcia-Pardo). Indeed, Garcia-Pardo implies that its 31-kDa fragment (shown in

Figure 3) is not even an Fn3 domain, let alone the tenth Fn3 domain, because Fn3 domains do not have disulfide bonds (present application at page 13, lines 4–5), yet the domain of Garcia-Pardo is suspected to contain disulfide bridges (page 10323, left column).

9. The PTO has also cited to evidence of mutational instability of proteins other than the tenth Fn3 domain of a mammalian fibronectin, citing to Mickle & Cutting, "Genotype–Phenotype Relationships in Cystic Fibrosis," *Med. Clin. North Am.* 84(3):597–607 (2000) for the example of cystic fibrosis transmembrane conductance regulator, DONALD VOET & JUDITH G. VOET, BIOCHEMISTRY126–128, 230 (1990) ("Voet") for the example of the hemoglobin beta subunit, and Yan et al., "Two–Amino Acid Molecular Switch in an Epithelial Morphogen that Regulates Binding to Two Distinct Receptors," *Science* 290(5491):523–527 (2000) for the example of ectodysplasin. The PTO has failed to demonstrate how these unrelated proteins are relevant to the tenth Fn3 domain of mammalian fibronectin. The recitation of isolated incidents of drastic mutational effects does not demonstrate that most (or even many) mutations have effects of such magnitude. Indeed, Voet states that mutations can change a protein "in ways that do not significantly affect its function," and teaches that critical residues and non-critical residues can be identified by comparing homologous proteins. DONALD VOET & JUDITH G. VOET, BIOCHEMISTRY 186 (3d ed. 2004) (attached hereto as Exhibit 2). Moreover, for the reasons discussed in paragraph 10 below, mutational effects are not unpredictable.

10. In the context of the present invention, mutational effects are not unpredictable for several reasons. First, the claimed invention relates to mutations of loop regions spanning between adjacent β-strands or to the N-terminal or C-terminal sequences. That is, the structural integrity of the β-strands present in the starting scaffold are maintained in the polypeptide monobodies. This allows the person of skill to expect the resulting mutant polypeptides to be structurally similar, while their binding activity to nuclear receptors may vary. The structure/function relationship is described at page 13, lines 26–27 (β-strands) and page 14, lines 5–9 (loop region sequence, N-terminal tail, and C-terminal tail) of the present application. Second, even if the resulting mutant polypeptides were structurally unsound (i.e., inherently unable to bind a nuclear receptor), the present application teaches how to perform a library selection to obtain only monobodies that exhibit nuclear receptor binding affinity. *See* Examples 1 and 2. In other words, if a fraction of monobodies in the library were non-functional, those non-functional monobodies would be screened out. Therefore, one of skill in

the art would know from the specification how to make and identify monobodies according to the claimed invention.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: March 29, 2006

Shohei Koide

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CLUSTAL W (1.82) multiple sequence alignment

                          A       B               C            D        E
HUMAN           VSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGETGGNSPVQEFTVPGSKSTATIS 60
MOUSE           VSDIPRDLEVIASTPTSLLISWEPPAVSVRYYRITYGETGGNSPVQEFTVPGSKSTATIN 60
CHIMPANZEE      VSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGETGGNSPVQEFTVPGSKSTATIS 60
DOG             VSDVPRDLEVIAATPTSLLISWDAPAVTVRYYRITYGETGGNSPVQEFTVPGSKSTATIS 60
CATTLE          VSDVPRDLEVIAATPTSLLISWDAPAVTVRYYRITYGETGGSSPVQEFTVPGSKSTATIS 60
RAT             VSDVPRDLEVIASTPTSLLISWEPPAVSVRYYRITYGETGGNSPVQEFTVPGSKSTATIN 60
                ***:******:*:*********:.***:*************.*****************.

                          F                   G
HUMAN           GLKPGVDYTITVYAVTGRGDSPASSKPISINYRT 94
MOUSE           NIKPGADYTITLYAVTGRGDSPASSKPVSINYKT 94
CHIMPANZEE      GLKPGVDYTITVYAVTGRGDSPASSKPISINYRT 94
DOG             GLKPGADYTITVYAVTGRGDSPASSKPVSIDYRT 94
CATTLE          GLKPGVDYTITVYAVTGRGDSPASSKPVSINYRT 94
RAT             NIKPGADYTITLYAVTGRGDSPASSKPVSINYQT 94
                .:***.*****:***************:**:*:*
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CLUSTAL W (1.82) Multiple Sequence Alignments

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Sequence format is Pearson
Sequence 1: HUMAN             94 aa
Sequence 2: MOUSE             94 aa
Sequence 3: CHIMPANZEE        94 aa
Sequence 4: DOG               94 aa
Sequence 5: CATTLE            94 aa
Sequence 6: RAT               94 aa
Start of Pairwise alignments
Aligning...
Sequences (1:2) Aligned. Score:  86
Sequences (1:3) Aligned. Score:  100
Sequences (1:4) Aligned. Score:  95
Sequences (1:5) Aligned. Score:  96
Sequences (1:6) Aligned. Score:  87
Sequences (2:3) Aligned. Score:  86
Sequences (2:4) Aligned. Score:  88
Sequences (2:5) Aligned. Score:  87
Sequences (2:6) Aligned. Score:  97
Sequences (3:4) Aligned. Score:  95
Sequences (3:5) Aligned. Score:  96
Sequences (3:6) Aligned. Score:  87
Sequences (4:5) Aligned. Score:  96
Sequences (4:6) Aligned. Score:  89
Sequences (5:6) Aligned. Score:  88
Guide tree        file created:   [/ebi/extserv/clustalw-work/interactive/clustalw-2
Start of Multiple Alignment
There are 5 groups
Aligning...
Group 1: Sequences:    2      Score:2020
Group 2: Sequences:    3      Score:2002
Group 3: Sequences:    4      Score:1995
Group 4: Sequences:    2      Score:2013
Group 5: Sequences:    6      Score:1932
Alignment Score 7929
CLUSTAL-Alignment file created  [/ebi/extserv/clustalw-work/interactive/clustalw-200
```

>HUMAN (Koide SEQ ID NO: 2) wil
VSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGETGGNSPVQEFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSPAS
>MOUSE (CAA63654) Fibronectin 9
VSDIPRDLEVIASTPTSLLISWEPPAVSVRYYRITYGETGGNSPVQEFTVPGSKSTATINNIKPGADYTITLYAVTGRGDSPAS
>CHIMPANZEE (XP_516072) Predict
VSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGETGGNSPVQEFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSPAS
>DOG (XP_536059) Predicted: sim
VSDVPRDLEVIAATPTSLLISWDAPAVTVRYYRITYGETGGNSPVQEFTVPGSKSTATISGLKPGADYTITVYAVTGRGDSPAS
>CATTLE (XP_879489) Predicted:
VSDVPRDLEVIAATPTSLLISWDAPAVTVRYYRITYGETGGSSPVQEFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSPAS
>RAT (NP_062016) Fibronectin 1
VSDVPRDLEVIASTPTSLLISWEPPAVSVRYYRITYGETGGNSPVQEFTVPGSKSTATINNIKPGADYTITLYAVTGRGDSPAS

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B. *Species Variations in Homologous Proteins: The Effects of Neutral Drift*

The primary structures of a given protein from related species closely resemble one another. If one assumes, according to evolutionary theory, that related species have evolved from a common ancestor, then it follows that each of their proteins must have likewise evolved from the corresponding protein in that ancestor.

A protein that is well adapted to its function, that is, one that is not subject to significant physiological improvement, nevertheless continues evolving. The random nature of mutational processes will, in time, change such a protein in ways that do not significantly affect its function, a process called **neutral drift** (deleterious mutations are, of course, rapidly rejected through natural selection). *Comparisons of the primary structures of* ***homologous proteins*** *(evolutionarily related proteins) therefore indicate which of the proteins' residues are essential to its function, which are of lesser significance, and which have little specific function.* If, for example, we find the same side chain at a particular position in the amino acid sequence of a series of related proteins, we can reasonably conclude that the chemical and/or structural properties of that so-called **invariant residue** uniquely suit it to some essential function of the protein. Other amino acid positions may have less stringent side chain requirements so that only residues with similar characteristics (e.g., those with acidic properties: Asp and Glu) are required; such positions are said to be **conservatively substituted.** On the other hand, many different amino acid residues may be tolerated at a particular amino acid position, which indicates that the functional requirements of that position are rather nonspecific. Such a position is called **hypervariable.**

a. Cytochrome *c* Is a Well-Adapted Protein

To illustrate these points, let us consider the primary structure of a nearly universal eukaryotic protein, **cytochrome *c*.** Cytochrome *c* has a single polypeptide chain that, in vertebrates, consists of 103 or 104 residues, but in other phyla has up to 8 additional residues at its N-terminus. It occurs in the mitochondrion as part of the **electron-transport chain,** a complex metabolic system that functions in the terminal oxidation of nutrients to produce adenosine triphosphate (ATP) (Section 22-2). The role of cytochrome *c* is to transfer electrons between a large enzyme complex known as **cytochrome *c* reductase** and one called **cytochrome *c* oxidase.**

It is believed that the electron-transport chain took its present form between 1.5 and 2 billion years ago as organisms evolved the ability to respire (Section 1-5C). Since that time, the components of this multienzyme system have changed very little, as is evidenced by the observation that the cytochrome *c* from any eukaryotic organism, say a pigeon, will react *in vitro* with the cytochrome oxidase from any other eukaryote, for instance, wheat. Indeed, hybrid cytochromes *c* consisting of covalently linked fragments from such distantly related species as horse and yeast (prepared via techniques of genetic engineering) exhibit biological activity.